

Institut für Parasitologie
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. med. vet. Peter Deplazes

Arbeit unter Leitung von Dr. sc. nat. Daniel Hegglin

**Stability of the southern European border of *Echinococcus*
multilocularis in the Alps: Evidence that *Microtus arvalis* is a limiting
factor**

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Diogo Ribeiro Almeida Guerra

Tierarzt
von Lissabon, Portugal

genehmigt auf Antrag von

Prof. Dr. med. vet. Peter Deplazes, Referent

Zürich 2014

INHALTSVERZEICHNIS

1. ZUSAMMENFASSUNG.....	1
-------------------------	---

2. SUMMARY	2
------------------	---

3. MANUSKRIFT	3 - 12
---------------------	--------

Stability of the southern European border of *Echinococcus multilocularis* in the Alps: Evidence that *Microtus arvalis* is a limiting factor

Parasitology (2014), doi:10.1017/S0031182014000730

4. DANKSAGUNG	
---------------	--

5. CURRICULUM VITAE	
---------------------	--

1. ZUSAMMENFASSUNG

Das Verbreitungsgebiet des zoonotischen Fuchsbandwurms *Echinococcus multilocularis* hat sich seit den 90er Jahren ausgeweitet, und der Parasit kann heute in weiten Teilen Europas häufiger festgestellt werden. Für dieses Phänomen werden hauptsächlich das Anwachsen der Europäischen Fuchspopulationen und deren Vordringen in den urbanen Raum verantwortlich gemacht. Jedoch reichen diese Faktoren allein nicht aus, um die heterogene Verbreitung des Parasiten in Europa zu erklären. In dieser Studie haben wir die räumliche Beziehung zwischen der Verbreitung von *E. multilocularis* und dem bekannten Vorkommen von sieben Wühlmausarten im Tessin analysiert. In 404 sezierten Füchsen (1990-2006) und 79 Fuchslosungen (2010-2012) wurde *E. multilocularis* fortwährend im Norden des Studiengebiets gefunden. Während den 22 Jahren des Untersuchungszeitraums wurde keine Ausweitung dieses endemischen Fokus festgestellt. Dieses stabile Vorkommen stimmt mit der bekannten Verbreitung der Wühlmaus *Microtus arvalis* aber nicht, oder nur teilweise, den Verbreitungsgebieten der anderen Wühlmausarten zusammen. Unsere Resultate weisen darauf hin, dass diese Wühlmausart eine zentrale Rolle für den Erhalt des Lebenszyklus des Parasiten spielen kann, und deren Absenz ein limitierender Faktor für die Ausbreitung von *E. multilocularis* in dieser Region darstellen könnte.

2. SUMMARY

The known range of the zoonotic fox tapeworm *Echinococcus multilocularis* has expanded since the 1990s, and today this parasite is recorded in higher abundances throughout large parts of Europe. This phenomenon is mostly attributed to the increasing European fox populations and their invasion of urban habitats. However, these factors alone are insufficient to explain the heterogeneous distribution of the parasite in Europe. Here, we analysed the spatial interrelationship of *E. multilocularis* with the known distribution of seven vole species in Ticino, southern Switzerland. Among 404 necropsied foxes (1990-2006) and 79 fox faecal samples (2010-2012), *E. multilocularis* was consistently found in the north of the investigated area. No expansion of this endemic focus was recorded during the 22 years of the study period. This stable endemic focus is coincident with the known distribution of the vole species *Microtus arvalis* but not, or only partly, with the distribution of the other autochthonous vole species. Our results give evidence that this vole species plays a crucial role in the maintenance of the parasite's life cycle and that its absence could be a limiting factor for the spread of *E. multilocularis* in this region.

Stability of the southern European border of *Echinococcus multilocularis* in the Alps: evidence that *Microtus arvalis* is a limiting factor

DIOGO GUERRA¹, DANIEL HEGGLIN¹, LUCA BACCIARINI²,
MANUELA SCHNYDER¹ and PETER DEPLAZES¹*

¹Institute of Parasitology, University of Zurich, Winterthurerstrasse 266a, CH-8057 Zurich, Switzerland

²Cantonal Veterinary Office, Via Dogana 16, CH-6500 Bellinzona, Switzerland

(Received 6 February 2014; revised 31 March 2014; accepted 1 April 2014)

SUMMARY

The known range of the zoonotic fox tapeworm *Echinococcus multilocularis* has expanded since the 1990s, and today this parasite is recorded in higher abundances throughout large parts of Europe. This phenomenon is mostly attributed to the increasing European fox populations and their invasion of urban habitats. However, these factors alone are insufficient to explain the heterogeneous distribution of the parasite in Europe. Here, we analysed the spatial interrelationship of *E. multilocularis* with the known distribution of seven vole species in Ticino, southern Switzerland. Among 404 necropsied foxes (1990–2006) and 79 fox faecal samples (2010–2012), *E. multilocularis* was consistently found in the north of the investigated area. No expansion of this endemic focus was recorded during the 22 years of the study period. This stable endemic focus is coincident with the known distribution of the vole species *Microtus arvalis* but not, or only partly, with the distribution of the other autochthonous vole species. Our results give evidence that this vole species plays a crucial role in the maintenance of the parasite's life cycle and that its absence could be a limiting factor for the spread of *E. multilocularis* in this region.

Key words: distribution, *Echinococcus multilocularis*, fox, *Microtus arvalis*, rodents, Switzerland.

INTRODUCTION

Echinococcus multilocularis is a small zoonotic tapeworm whose life cycle is based on a predator–prey interaction. In Europe, the red fox (*Vulpes vulpes*) is the main definitive host, harbouring the parasite's adult stage in the small intestine. Different rodents are infected upon ingesting eggs from a contaminated environment and the subsequent development of an alveolar metacestode in the liver (Eckert *et al.* 2011). Arvicolid, especially the common vole (*Microtus arvalis*) and the water vole (*Arvicola scherman*, former *Arvicola terrestris*), are considered as the main intermediate hosts in Europe (Houin *et al.* 1982; Stieger *et al.* 2002; Reperant *et al.* 2009).

Echinococcus multilocularis is distributed over large regions throughout the northern hemisphere (Eckert *et al.* 2011). Its historical endemic area in Europe was circumscribed to eastern France, Switzerland, southern Germany and western Austria (Rausch, 1967), but during the last three decades, *E. multilocularis* infections in foxes have been reported far outside this region from western France (Combes *et al.* 2012) to Romania (Sikó *et al.* 2011), Ukraine (Kharchenko *et al.* 2008), the Baltic countries (Moks

et al. 2005; Bružinskaitė *et al.* 2007) and as far north as southern Sweden (Lind *et al.* 2011). Such new records not necessarily document a real spread as the detection probability in low endemic areas strongly depends on the sampling effort. In Sweden, for example, 2985 foxes shot in 2011 had to be analysed in order to detect three positive animals in three very distinct foci (Lind *et al.* 2011). Therefore, it is hard to judge whether these records reproduce an expansion or just the first findings in very low endemic areas. However, existing data clearly show that *E. multilocularis* in foxes became at least more abundant over a large part of Europe during the last two decades (Sreter *et al.* 2003; Combes *et al.* 2012).

In this study, the southern border of *E. multilocularis* in Europe is the focus (Fig. 1). The most southern *E. multilocularis* records in foxes in France are reported in the historically endemic department of Cantal (Deblock *et al.* 1988) and recently further east, in the department of Savoie (Combes *et al.* 2012). South of the Alps, infected foxes have already been recorded in the cantons of Ticino (Ewald, 1993) and Grisons, Switzerland (Tanner *et al.* 2006), and in the very northern part of Italy, in Bolzano and Trento provinces (Manfredi *et al.* 2002; Casulli *et al.* 2005). The *E. multilocularis* endemic areas in northern Italy are adjacent to the Austrian ones, where the parasite seems to be ubiquitously

* Corresponding author: Institute of Parasitology, Winterthurerstrasse 266a, CH-8057 Zurich, Switzerland. E-mail: deplazes@access.uzh.ch



Fig. 1. Distribution of the most southern infections by *Echinococcus multilocularis* in foxes from the Alps and adjacent regions. AT – Austria; CH – Switzerland; DE – Germany; FR – France; HR – Croatia; IT – Italy; SI – Slovenia. References: 1 – Combes *et al.* (2012); 2 – Ewald (1993); 3 – Tanner *et al.* (2006); 4 – Duscher *et al.* (2006); 5 – Casulli *et al.* (2005); 6 – Rataj *et al.* (2010). The dashed line represents the main Alpine divide.

distributed (Duscher *et al.* 2006). No records exist further south in the Italian peninsula (Di Cerbo *et al.* 2008; Magi *et al.* 2009). Towards east and southeast, the parasite is known to be present in Slovenia (Rataj *et al.* 2010), Hungary (Sreter *et al.* 2003) and in adjacent areas in north-western Romania (Sikó *et al.* 2011). No foxes were found infected from the north of Croatia (Rajković-Janje *et al.* 2002), but the presence of *E. multilocularis* metacestodes is described in *M. arvalis* from the north of Bulgaria (Genov *et al.* 1980).

As mentioned above, abundance and prevalence of *E. multilocularis* in foxes were shown to have augmented over large areas, e.g. the Netherlands (Takumi *et al.* 2008) or Germany (Berke *et al.* 2008; Staubach *et al.* 2011), respectively. Likewise, a growing incidence is reported for alveolar echinococcosis (AE) in humans. In Switzerland, for example, the incidence of human cases increased 2.5 times between the years 2000–2005 (Schweiger *et al.* 2007). A tendency in the increase of AE cases was also documented for Austria (Schneider *et al.* 2013) and France (Said-Ali *et al.* 2013). The rising numbers of foxes and the urbanization of the *E. multilocularis* life cycle in Europe have been pointed out as possible causes for the described patterns (Deplazes *et al.* 2004; Fischer *et al.* 2005; Schweiger *et al.* 2007).

In Belgium, on the other hand, no emergence of the parasite was detected in foxes during 1996–2008, although the fox population had considerably increased during the same period (Van Gucht *et al.* 2010). This suggests that the observed trends for *E. multilocularis* in Europe are heterogeneous and can only partially be explained by changes in the fox population dynamics.

The spatial dynamics of *E. multilocularis* occurrence and abundance are not well understood and few studies investigated different factors putatively limiting the distribution of this parasite. Mean temperature and humidity, for example, have an impact on egg survival in the environment (Veit *et al.* 1995) and may affect the transmission potential and the infection of intermediate hosts. However, it has to be considered that the role of these hosts depends not only on their infection rates, but also on the fertility of the larval stages and on the extent by which they are predated by definitive hosts. Moreover, the distribution and abundance of intermediate hosts are likewise expected to be key factors for the establishment and maintenance of the life cycle in a given habitat (Giraudoux *et al.* 2003; Hansen *et al.* 2004; Guislain *et al.* 2008; Raoul *et al.* 2010). The distribution of rodent species is mainly shaped by the availability of suitable habitats and by climatic factors

Table 1. *Echinococcus multilocularis* in foxes ($n = 404$) and fox faecal samples ($n = 79$) from the Canton Ticino (Switzerland) during 1990–2012

Period	Diagnostic techniques ^a	<i>N</i> total	Nendemic area	<i>E. multilocularis</i> prevalence (CI 95%) endemic area	<i>E. multilocularis</i> prevalence (CI 95%) non-endemic area
1990–1992 ^b	IST	55	15	13.3 (1.7–40.5)	0.0 (0.0–7.2)
1993–1994 ^c	IST	93	27	7.4 (0.9–24.3)	0.0 (0.0–4.4)
1999–2000	SCT	75	24	4.2 (0.1–21.1)	0.0 (0.0–5.7)
2002–2003	SCT	57	47	10.6 (3.5–23.1)	0.0 (0.0–25.9)
2005–2006	SCT	124	33	6.1 (0.7–20.2)	0.0 (0.0–3.2)
2010–2012	Coprology	79	16	6.3 (0.2–30.2)	0.0 (0.0–4.6)

^a IST – Intestinal Scraping Technique; SCT – Sedimentation and Counting Technique; Coprology – Sieving and flotation technique and molecular identification.

^b Ewald (1993).

^c Alther (1996).

(Giraudoux *et al.* 2003, 2013b) as well as by post-glacial range expansion (Braaker and Heckel, 2009). Accordingly, a recent study in China demonstrated how key rodent species could be used to describe the distribution ranges of *E. multilocularis* over large areas (Giraudoux *et al.* 2013a). However, the distribution of most rodents is rather heterogeneous and rodent community composition can be highly variable over small areas, just like the patchy distribution of *E. multilocularis* infections in rodents and foxes (Tanner *et al.* 2006). In the framework of this study, we investigated the long-term spatial dynamics of *E. multilocularis* on a small scale in the south of Switzerland and analysed how the observed pattern correlates with the known distribution of autochthonous vole species.

MATERIALS AND METHODS

Study area

This study was conducted in the Canton of Ticino (southern Switzerland) which has a surface area of approximately 2812 km². Half of the territory is covered by forest, 30% by unproductive areas (i.e. mountains, lakes and rivers), 13% by agricultural areas and 6% by human infrastructures (data: Swiss Federal Statistics Office, www.bfs.admin.ch). There is a predominance of an alpine landscape, with deep valleys and high mountains (altitudes range from 200 to 3400 m).

There are seven Arvicolid species described in Ticino, which are potential intermediate hosts for *E. multilocularis*: *Arvicola amphibius*, *Chionomys nivalis*, *M. arvalis*, *Microtus multiplex*, *Microtus savii*, *Microtus subterraneus* and *Myodes glareolus* (Hausser, 1995).

Samples

A total of 404 red foxes, obtained from hunters between 1990 and 2006, were analysed. These specimens had been shot in the course of the official hunting seasons and a small percentage (<2%)

had been found dead (e.g. road killed). Five time periods were studied: 1990–1992 (Ewald, 1993); 1993–1994 (Alther, 1996); 1999–2000; 2002–2003 and 2005–2006 (unpublished results). Sex, age and location were recorded for each animal. Whenever the exact location was not available, the coordinates of the nearest human settlement were used. In order to inactivate taeniid eggs, carcasses were deep-frozen at –80 °C for at least 5 days (Eckert *et al.* 2001). Helminthological investigations were performed either by the Intestinal Scraping Technique (IST) (Eckert *et al.* 2001) or by the Sedimentation and Counting Technique (SCT) (Hofer *et al.* 2000) (Table 1).

Based on typical morphological characteristics, prevalence rates of some common intestinal helminths were determined: *E. multilocularis* (for the period 1990–2006), *Taenia* spp., *Mesocostoides* spp., hookworms (*Uncinaria* spp.) and ascarids (*Toxocara* spp. and *Toxascaris leonina*) (for the period 1999–2006 only).

During 2010–2012, 79 fox and 23 dog faecal samples were collected in the same study area. Collection was performed between April and November when snow or grass coverage were lowest. Species identification for faecal samples was based on content, morphology, odour and location (Stieger *et al.* 2002). Faecal samples were also frozen at –80 °C for at least 5 days prior to any analysis. Two grams of each sample were screened for taeniid eggs with a sieving-flotation technique (Mathis *et al.* 1996). DNA extraction from positive samples was performed according to Štefanić *et al.* (2004) and a multiplex-PCR for taeniid genus identification was used (Trachsel *et al.* 2007), with the primer pairs described by the authors. *Echinococcus multilocularis* positive samples were confirmed by sequencing, after purification with a MinElute PCR purification kit (Qiagen, Hilden, Germany). Sequencing was carried out by Synergene Biotech GmbH, Biotech Centre Zurich, Switzerland (www.synergene-biotech.com) and results compared with GenBank nucleotide database (BLAST; www.blast.ncbi.nlm.nih.gov).

Spatial and statistical analysis

A map with the coordinates of all carcasses and faecal samples was built using the software QuantumGIS version 1.8.0 Lisboa, <http://qgis.org/>. Official Switzerland borders were obtained from the Swiss Federal Office of Topography (www.swisstopo.admin.ch/; version from 1.1.2013).

The Swiss Biological Records Center (CSCF) (<http://lepus.unine.ch/carto/>) provides cartographical server information on the known distribution of the Swiss fauna. The distribution maps of the different rodent species, which are provided on the base of a 5 × 5 km grid, were used for comparisons with the recorded distribution of *E. multilocularis* in foxes.

The endemic area for *E. multilocularis* was defined by all grid cells where infections of fox origin were recorded and a buffer zone of 1 cell (5 × 5 km) around this area therewith accounting for the spatial behaviour of foxes. Fox home-range sizes in different studies on Continental Europe ranged between 0.6–9.3 km² (Trehwella *et al.* 1988; Meia and Weber, 1995). All grid cells outside this area were referred as belonging to a non-endemic area.

As rough indicators of fox predation on rodents, prevalence rates of rodent- and non-rodent-related intestinal helminths were compared between foxes originating from the *E. multilocularis* endemic and non-endemic areas, using the Chi-square test. Statistical analysis was carried out using SPSS 20.0. Significance value was set as $P < 0.05$.

RESULTS

Between one and five *E. multilocularis* positive samples were recorded in foxes in every period studied (Table 1). The periodic prevalence rates in the endemic area ranged between 4.2 and 13.3% and the overall rates were 9.5% (IST; 1990–1994) and 7.7% (SCT; 1999–2006). The occurrence of *E. multilocularis* in fox faecal samples was 6.3% (2010–2012). The presence of *E. multilocularis* eggs was detected in one out of just four dog faecal samples from the endemic area. For this faecal sample only, the host species was confirmed by a multiplex-PCR (Nonaka *et al.* 2009).

All positive *E. multilocularis* samples ($n = 13$) were from an endemic area with approximately 160 km² in the most northern part of Ticino (Fig. 2), just south of the main Alpine divide. In this area there are two main valleys with north-south orientation that merge further south: Val Leventina in the West and Valle di Blenio in the East (Fig. 2). Most of the positive samples were from Val Leventina and only one from Valle di Blenio. No evident changes in the latitude of the infections were recorded throughout the study periods (Fig. 2). The most southern infected fox was located at latitude 46.49°N (Decimal degrees,

WGS84) in Val Leventina. The positive dog sample originated from approximately 2 km southwest from this point.

Analysis of rodent communities revealed that *M. arvalis* is the only species contemporaneously present in the *E. multilocularis* endemic area and completely absent in the other area (Fig. 3A). *Microtus subterraneus* was also predominantly recorded in the endemic area, but there are records in two other locations further south (Fig. 3F). The distribution of all other Arvicolids was apparently unrelated to the one of *E. multilocularis* in foxes, including *A. amphibius* and *M. savii* which were present solely in the non-endemic area.

There was a significantly higher prevalence of *Taenia* species in the *E. multilocularis* endemic area (Fig. 4). The prevalence rates of *Mesocostoides* spp., hookworms and ascarids exhibited no differences between the two investigated areas.

DISCUSSION

Distribution of E. multilocularis in foxes

Our results give no evidence for a spread of the distribution of *E. multilocularis* in Ticino over a 20-year period. The infected samples were constantly obtained from a very small geographic area and there were no major changes in their latitude that could suggest a southern spread.

The fact that all 258 foxes investigated from southern areas of Ticino were not infected gives strong evidence that the parasite is absent in this region or only occurs occasionally.

The overall *E. multilocularis* prevalence in foxes from the endemic area in Ticino is much lower compared with hyper-endemic locations north of the Alps, where prevalence rates higher than 30% have frequently been recorded, e.g. in Switzerland (Brossard *et al.* 2007; Hegglin *et al.* 2007; Reperant *et al.* 2007) or Austria (Duscher *et al.* 2006). In fact, the prevalence rate is similar to the ones obtained from other alpine regions, such as the Swiss canton of Grisons (< 14.3%) (Tanner *et al.* 2006) or the Bolzano and Trento provinces, in northern Italy (< 12.9%) (Manfredi *et al.* 2002; Casulli *et al.* 2005) (Fig. 1). A north-to-south decreasing gradient of the prevalence rates is evident between the highly endemic areas in northern Switzerland and Austria, and adjacent foci in the South. On these three alpine regions, infected foxes exhibit a patchy distribution coincident with specific valleys. In the Canton of Grisons, infected foxes have been found in the Müstair Valley that is in close connection with an Italian valley in Bolzano, where one of the Italian foci is located (Casulli *et al.* 2009). Since foxes can disperse over large distances (Trehwella *et al.* 1988; Meia and Weber, 1995), occasional exchanges of parasites between these two areas are likely.

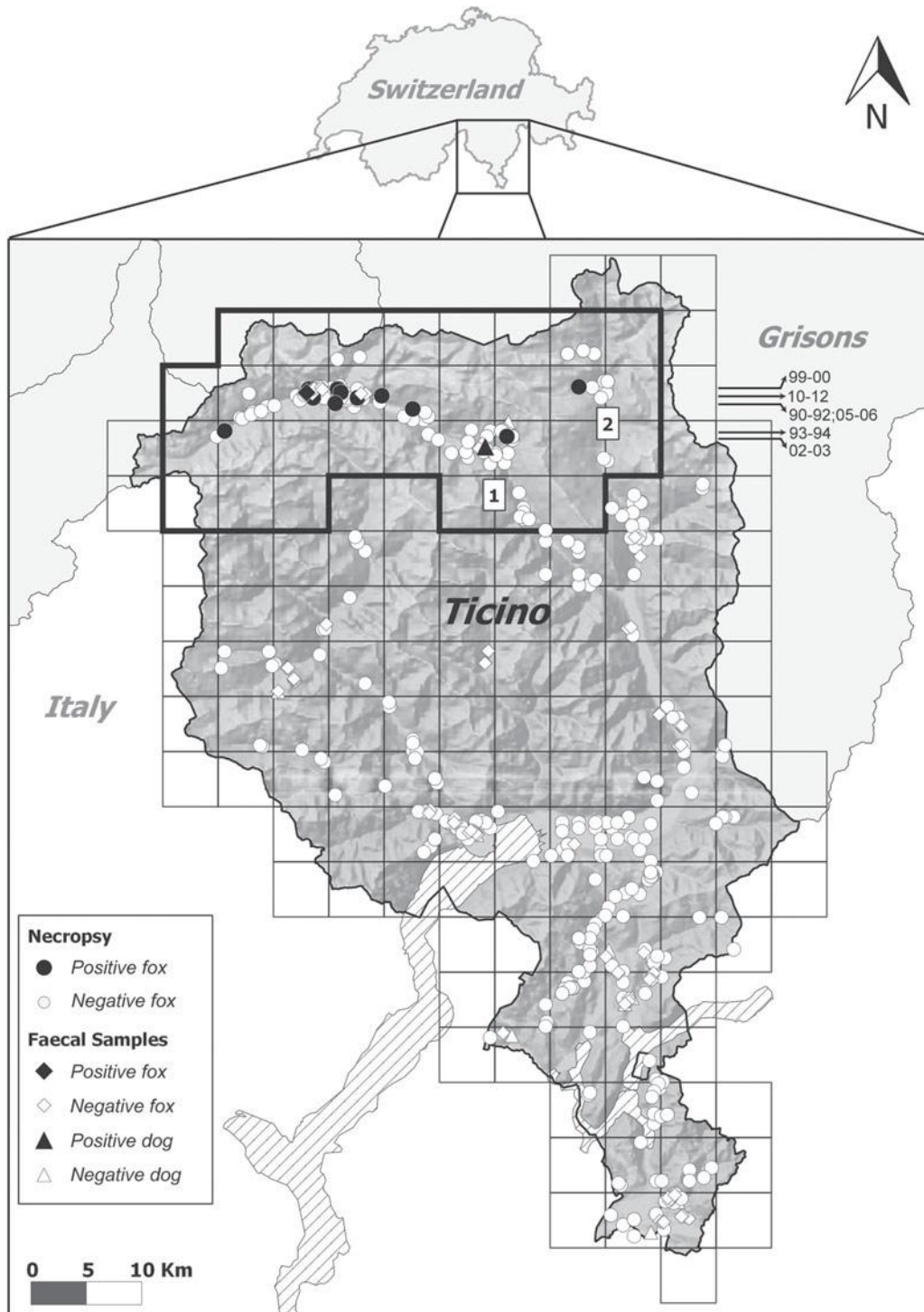


Fig. 2. *Echinococcus multilocularis* in foxes and dogs from the Canton of Ticino (southern Switzerland) between 1990 and 2012. Grid with 5×5 km cells. The bold gridlines define the endemic area of *E. multilocularis*. 1 – Val Leventina; 2 – Valle di Blenio. The arrows in the upper right corner depict the latitude of the most southern case in a fox during each studied period.

The first *E. multilocularis* infections in foxes from Ticino were recorded more than 20 years ago (Ewald, 1993) but no autochthonous human cases have been documented in this region so far.

Autochthonous human AE was reported in two patients from South Tyrol between 1906 and 1922 (Hosemann *et al.* 1928) nearby the location of reported infections in foxes from northern Italy.

This may shed some light on the age and dynamics of these different alpine *E. multilocularis* foci that seem rather stable and not a result of a recent spread.

Rodent species distribution and fox predation on rodents

The analysis of the distribution areas of the vole species in Ticino gives evidence that *M. arvalis* is

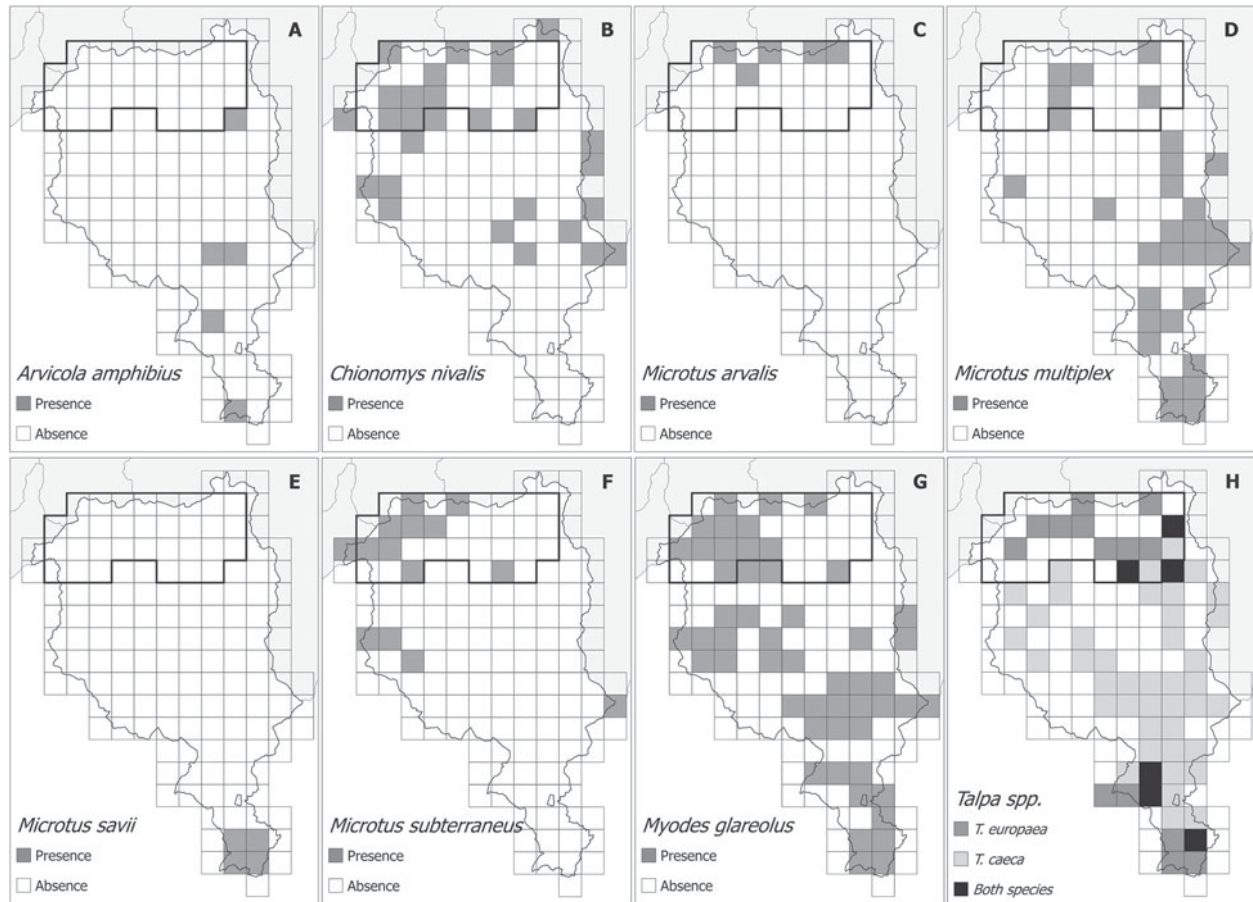


Fig. 3. Presence/absence of rodent (A–G) and insectivore (H) species in Ticino (Switzerland). Data obtained from the online cartographical server of the Swiss Biological Records Center (<http://lepus.unine.ch/carto/>). Grid with 5 x 5 km cells. Bold gridlines define the endemic area for *Echinococcus multilocularis* in Ticino (see Fig. 2).

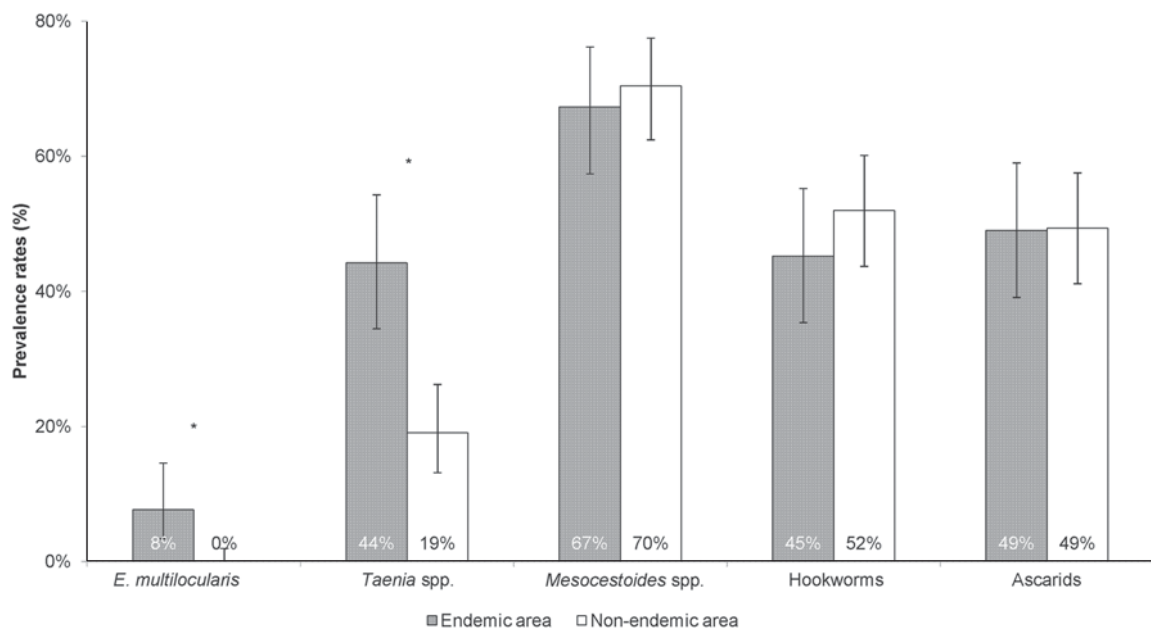


Fig. 4. Comparison of helminth prevalence rates between foxes from the *Echinococcus multilocularis* endemic ($n=104$) and non-endemic ($n=152$) areas in Ticino during 1999–2006. Asterisks: $P<0.05$ (Chi-square test); error bars: 95% confidence interval.

likely to act as the most important intermediate host for *E. multilocularis* in this Canton. Its apparent absence from the non-endemic area may be a limiting factor for the parasite's spread. Other rodent species present in both areas may also act as intermediate hosts. *Microtus subterraneus* is mostly found in the *E. multilocularis* endemic area but also in a few locations in the non-endemic area. There is a record of *E. multilocularis* infection in this species, i.e. one out of 169 necropsied specimens in France (Delattre *et al.* 1990), but no other extensive studies have been carried out so far. Although *M. subterraneus* is not a very abundant vole (Hausser, 1995), the number of foxes collected from these locations in the non-endemic area is too small to completely discard its relevance as an intermediate host. Conversely, *M. glareolus*, which has been described as a potential intermediate host for *E. multilocularis* (Bonnin *et al.* 1986; Stieger *et al.* 2002) has a distribution that is clearly unrelated to *E. multilocularis* infections in foxes. Maybe due to low densities or because they are not as important in foxes' diet, the presence of this and of the other rodent species in the non-endemic area seems to be insufficient to maintain the parasite's life cycle. The former species *A. terrestris*, an important intermediate host for *E. multilocularis*, has been reclassified into *A. scherman* and *A. amphibius* (Wilson and Reeder, 2005). *Arvicola scherman*, absent in Ticino, is abundant in *E. multilocularis* highly endemic areas. For example, in Zurich, Switzerland, high prevalence rates of infection (up to 40.6–78.5% in some areas) with fertile metacystodes (overall 9.3%) have been documented in this species (Burlet *et al.* 2011). Interestingly, in our study *A. amphibius* was located exclusively in the *E. multilocularis* non-endemic area in Ticino. In contrast to *A. scherman*, this species reaches regions far outside the known endemic area of *E. multilocularis* in Europe. It is a semi-aquatic species associated with wetlands, rivers and ponds (Hausser, 1995) and this environment may protect it from fox predation. While *A. scherman* can be considered an important intermediate host, according to our study, the relevance of *A. amphibius* for the *E. multilocularis* life cycle in this region is questionable.

In the Massif Central, France, in an area of roughly 5000 km², Deblock *et al.* (1988) defined a border between an endemic and a non-endemic area for *E. multilocularis*, based on the necropsies of foxes. This endemic area corresponded to locations where infected *Arvicola* sp. were previously recorded. Unfortunately, the authors did not describe the distribution of the other rodent species which derails possible relationships between the distribution of potential intermediate hosts and the observed pattern for *E. multilocularis* in foxes.

Another key factor to understand the role of different intermediate hosts in the parasite's life cycle is the fox predation rate. Foxes exhibit a dietary plasticity

that is nonetheless related to a preference for certain prey (Macdonald, 1977; Hegglin *et al.* 2007). In Zurich, Switzerland, although burrow systems of *M. arvalis* were far less frequently recorded than the ones from *A. scherman*, the frequency of both species' remains in fox stomachs was similar (Hegglin *et al.* 2007). In other highly endemic areas for *E. multilocularis* in France, *M. arvalis* was the most common prey (Guislain *et al.* 2008) and foxes exhibited a predatory preference for it (Raoul *et al.* 2010). In the Münstair Valley, where no *Arvicola* species are recorded, *E. multilocularis* in foxes was likely associated with predation on *Microtus* species (Tanner *et al.* 2006). However, a study in western Switzerland showed that *A. scherman* can also be the most common prey of foxes (Weber and Aubry, 2009) and is likely to act as a key species for the parasite's transmission in certain areas. Unlike other less predated Arvicolids, *A. scherman* and *M. arvalis* are known agricultural pests. They inhabit meadows and pastures and develop pluriannual population cycles, reaching as many as 1000 and 2500 individuals ha⁻¹, respectively (Hausser, 1995).

The analysis of other helminths' frequency in foxes may deliver some more information on their diet. Interestingly, *Taenia* spp. occurred more frequently in foxes from the *E. multilocularis* endemic area (Fig. 4). *Taeniocrassiceps* and *Taeniapolyacantha* are the most common *Taenia* species in foxes from Ticino (Ewald, 1993). Like *E. multilocularis*, both species have a dioxenous life cycle, in which rodents are the most important intermediate hosts. *Microtus arvalis* is considered the most susceptible intermediate host for *T. crassiceps* (Rietschel, 1981) and is also an intermediate host for *T. polyacantha* (Jones and Pybus, 2001). In the Canton of Thurgau, Switzerland, a study on helminths of rodent species succeeded in finding both *T. polyacantha* and *T. crassiceps* in *M. arvalis* (Schaerer, 1987). There were significant differences between this species and *M. glareolus*, in which no infections were found. The higher number of *Taenia* spp. infections in the *E. multilocularis* endemic area may be related to the availability of susceptible intermediate hosts, such as *M. arvalis*, thus reinforcing its relevance in the foxes' diet and in the *E. multilocularis* life cycle. In contrast, helminths with life cycles not dependent on microtine species exhibited no spatial segregation.

Limiting factors for the spread of E. multilocularis

Geographic barriers have been impacting the spread and distribution of rodents for a long time (Braaker and Heckel, 2009). After the Last Glacial Maximum (LGM), which took place more than 200 000 years ago, animal and plant species were able to recolonize previously frozen regions (Sommer and Nadachowski, 2006). The current distribution of these species was strongly affected by the outcome of

these events. The migration patterns of foxes and intermediate hosts after the LGM and the gradual colonization of different areas may help in the understanding of the current distribution of *E. multilocularis* and the patchy arrangement of infected foxes in the Alps. In a work by Braaker and Heckel (2009), mitochondrial DNA of different *M. arvalis* isolates revealed that the post-glaciation migrations of this species might have occurred upwards from Italy through the valleys of the bigger rivers in Switzerland. It is surprising to see that the postulated main routes of migration overlap with the valleys where *E. multilocularis* was found in Ticino (this paper) and in Grisons (Engadin and Bregaglia valleys) (Tanner *et al.* 2006). These findings seem to reinforce the hypothesis of *M. arvalis* relevance for *E. multilocularis* in these regions or at least reflect areas where the contact between definitive and intermediate hosts has occurred long enough to allow the establishment of a parasitic life cycle.

Analysis of the distribution of other species can help clarify the impact of geographic barriers. *Talpa caeca* and *Talpa europaea* are insectivores that often share the habitat with *M. arvalis* and *A. terrestris* (Giraudoux *et al.* 2003; Delattre *et al.* 2006). In Ticino, these two *Talpa* species have a segregated distribution (Fig. 3H). Maddalena *et al.* (2000) documented a clear border in Val Leventina, mostly due to geographic barriers. This border was set around latitude 46°42'N coincident with the border of the endemic area for *E. multilocularis* obtained in the present study. Since *M. arvalis* is a grassland rodent, it is reasonable to assume that in Val Leventina the border features for the *Talpa* species would also be valid for *M. arvalis*, preventing it from spreading further south. If *M. arvalis* is the most important intermediate host for *E. multilocularis* in Ticino, its circumscription to the north of the canton would prevent the parasite from establishing on more southern areas where no key intermediate host is present.

Climatic variables in Ticino, such as temperature and rainfall, are distinct between the very northern mountain valleys and the more temperate lakeside pastures in the south. This north-south gradient in temperature and humidity could act as an adjuvant in balancing the stable epidemiological situation for *E. multilocularis*. However, as seen in Fig. 1, foxes have been found infected even in regions south from Ticino. This shows that climate is not *per se* an absolute exclusion factor for the distribution of the parasite in Europe and that other variables, such as the distribution of rodent communities, should be henceforth more frequently considered.

ACKNOWLEDGEMENTS

To Tiziano Maddalena, Simon Capt and Jürg Paul Müller for the valuable comments on the rodents' distribution in

Ticino. Also, to Laura Lurati for collecting part of the faecal samples and to Alexander Mathis for comments on the manuscript. This study represents the dissertation of Diogo Guerra, veterinarian.

FINANCIAL SUPPORT

This work was supported by the Federal Food Safety and Veterinary Office (FSVO) of Switzerland, by the EMIDA-ERA NET framework, and is within the scope of the EMIRO project 'The significance of rodent communities for the distribution of *Echinococcus multilocularis*: ecological and experimental investigations' (grant number 1.12.18 EMIDA EMIRO).

REFERENCES

- Alther, P. (1996). *Beitrag zur Epidemiologie und Diagnose der Echinococcus multilocularis – Infektion bei Endwirten*. Vet Med thesis. University of Zurich, Zurich, Switzerland.
- Berke, O., Romig, T. and von Keyserlingk, M. (2008). Emergence of *Echinococcus multilocularis* among red foxes in northern Germany, 1991–2005. *Veterinary Parasitology* 155, 319–322.
- Bonnin, J., Delattre, P., Artois, M., Pascal, M., Aubert, M. and Petavy, A. (1986). Contribution à la connaissance des hôtes intermédiaires d'*Echinococcus multilocularis* dans le nord-est de la France. *Annales de Parasitologie Humaine et Comparée* 61, 235–243.
- Braaker, S. and Heckel, G. (2009). Transalpine colonisation and partial phylogeographic erosion by dispersal in the common vole (*Microtus arvalis*). *Molecular Ecology* 18, 2518–2531.
- Brossard, M., Andreutti, C. and Siegenthaler, M. (2007). Infection of red foxes with *Echinococcus multilocularis* in western Switzerland. *Journal of Helminthology* 81, 369–376.
- Bružinskaitė, R., Marcinkutė, A., Strupas, K., Sokolovas, V., Deplazes, P., Mathis, A., Eddi, C. and Šarkūnas, M. (2007). Alveolar echinococcosis, Lithuania. *Emerging Infectious Diseases* 13, 1618–1619.
- Burlet, P., Deplazes, P. and Hegglin, D. (2011). Age, season and spatio-temporal factors affecting the prevalence of *Echinococcus multilocularis* and *Taenia taeniaeformis* in *Arvicola terrestris*. *Parasites and Vectors* 4, 1–9.
- Casulli, A., Manfredi, M. T., La Rosa, G., Di Cerbo, A. R., Dinkel, A., Romig, T., Deplazes, P., Genchi, C. and Pozio, E. (2005). *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) of the Italian Alpine region: is there a focus of autochthonous transmission? *International Journal for Parasitology* 35, 1079–1083.
- Casulli, A., Bart, J. M., Knapp, J., La Rosa, G., Dusher, G., Gottstein, B., Di Cerbo, A., Manfredi, M. T., Genchi, C., Piarroux, R. and Pozio, E. (2009). Multi-locus microsatellite analysis supports the hypothesis of an autochthonous focus of *Echinococcus multilocularis* in northern Italy. *International Journal for Parasitology* 39, 837–842.
- Combes, B., Comte, S., Raton, V., Raoul, F., Boué, F., Umhang, G., Favier, S., Dunoyer, C., Woronoff, N. and Giraudoux, P. (2012). Westward spread of *Echinococcus multilocularis* in foxes, France, 2005–2010. *Emerging Infectious Diseases* 18, 2059–2062.
- Deblock, S., Pétavy, A. F. and Gilot, B. (1988). Helminthes intestinaux du renard commun (*Vulpes vulpes* L.) dans le Massif central (France). *Canadian Journal of Zoology* 66, 1562–1569.
- Delattre, P., Giraudoux, P. and Quéré, J.-P. (1990). Conséquences épidémiologiques de la réceptivité d'un nouvel hôte intermédiaire du *Taenia multiloculaire* (*Echinococcus multilocularis*) et de la localisation spatiotemporelle des rongeurs infestés. *Comptes rendus de l'Académie des Sciences* 310, 339–344.
- Delattre, P., Clarac, R., Melis, J.-P., Pleydell, D. and Giraudoux, P. (2006). How moles contribute to colonization success of water voles in grassland: implications for control. *Journal of Applied Ecology* 43, 353–359.
- Deplazes, P., Hegglin, D., Gloor, S. and Romig, T. (2004). Wilderness in the city: the urbanization of *Echinococcus multilocularis*. *Trends in Parasitology* 20, 77–84.
- Di Cerbo, A. R., Manfredi, M. T., Trevisiol, K., Bregoli, M., Ferrari, N., Pirinesi, F. and Bazzoli, S. (2008). Intestinal helminth communities of the red fox (*Vulpes vulpes* L.) in the Italian Alps. *Acta Parasitologica* 53, 302–311.
- Duscher, G., Pleydell, D., Prosl, H. and Joachim, A. (2006). *Echinococcus multilocularis* in Austrian foxes from 1991 until 2004. *Journal of Veterinary Medicine, Series B* 53, 138–144.

- Eckert, J., Gemmell, M., Meslin, F. and Pawlowski, Z. (2001). *WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern*, Vol. 32. World Organisation for Animal Health, Paris, France.
- Eckert, J., Deplazes, P. and Kern, P. (2011). Alveolar echinococcosis (*Echinococcus multilocularis*) and neotropical forms of echinococcosis (*Echinococcus vogeli* and *Echinococcus oligarthrus*). In *Oxford Textbook of Zoonoses: Biology, Clinical Practice, and Public Health Control*, 2nd Edn (ed. Palmer, S.R., Soulsby, E.J.L., Torgerson, P.R. and Brown, D.W.G.), pp. 668–699. Oxford University Press, Oxford, UK.
- Ewald, D. (1993). *Prävalenz von Echinococcus multilocularis bei Rotfüchsen (Vulpes vulpes L.) in der Nord-, Ost- und Südschweiz sowie im Fürstentum Liechtenstein*. Ph.D. thesis, Phil. II. University of Zurich, Zurich, Switzerland.
- Fischer, C., Reperant, L., Weber, J., Hegglin, D. and Deplazes, P. (2005). *Echinococcus multilocularis* infections of rural, residential and urban foxes (*Vulpes vulpes*) in the canton of Geneva, Switzerland. *Parasite* 12, 339–346.
- Genov, T., Svilenov, D. and Polyakova-Krusteva, O. (1980). The natural occurrence of *Alveococcus multilocularis* in the *Microtus nivalis* in Bulgaria. *Doklady Bolgarskoi Akademii Nauk* 33, 981–984.
- Giraudoux, P., Craig, P., Delattre, P., Bao, G., Bartholomot, B., Harraga, S., Quéré, J., Raoul, F., Wang, Y. and Shi, D. (2003). Interactions between landscape changes and host communities can regulate *Echinococcus multilocularis* transmission. *Parasitology* 127, S121–S132.
- Giraudoux, P., Raoul, F., Afonso, E., Ziadinov, I., Yang, Y., Li, L., Li, T., Quéré, J.-P., Feng, X. and Wang, Q. (2013a). Transmission ecosystems of *Echinococcus multilocularis* in China and Central Asia. *Parasitology* 140, 1655–1666.
- Giraudoux, P., Raoul, F., Pleydell, D., Li, T., Han, X., Qiu, J., Xie, Y., Wang, H., Ito, A. and Craig, P.S. (2013b). Drivers of *Echinococcus multilocularis* transmission in china: small mammal diversity, landscape or climate? *PLOS Neglected Tropical Diseases* 7, e2045. doi: 10.1371/journal.pntd.0002045.
- Guislain, M.-H., Raoul, F., Giraudoux, P., Terrier, M.-E., Froment, G., Ferté, H. and Pouille, M.-L. (2008). Ecological and biological factors involved in the transmission of *Echinococcus multilocularis* in the French Ardennes. *Journal of Helminthology* 82, 143–151.
- Hansen, F., Jeltsch, F., Tackmann, K., Staubach, C. and Thulke, H.-H. (2004). Processes leading to a spatial aggregation of *Echinococcus multilocularis* in its natural intermediate host *Microtus arvalis*. *International Journal for Parasitology* 34, 37–44.
- Hausser, J. (1995). *Säugetiere der Schweiz*. Birkhäuser, Basel, Switzerland.
- Hegglin, D., Bontadina, F., Contesse, P., Gloor, S. and Deplazes, P. (2007). Plasticity of predation behaviour as a putative driving force for parasite life-cycle dynamics: the case of urban foxes and *Echinococcus multilocularis* tapeworm. *Functional Ecology* 21, 552–560.
- Hofer, S., Gloor, S., Müller, U., Mathis, A., Hegglin, D. and Deplazes, P. (2000). High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zurich, Switzerland. *Parasitology* 120, 135–142.
- Hosemann, G., Schwarz, E., Lehmann, J. C. and Posselt, A. (1928). Die Echinokokken Krankheit. In *Neue Deutsche Chirurgie*, Vol. 40 (ed. Küttner, H.), pp. 103–107. Ferdinand Enke Verlag, Stuttgart, Germany.
- Houin, R., Deniau, M., Liance, M. and Puel, F. (1982). *Arvicola terrestris*, an intermediate host of *Echinococcus multilocularis* in France: epidemiological consequences. *International Journal for Parasitology* 12, 593–600.
- Jones, A. and Pybus, M.J. (2001). Taeniasis and echinococcosis. In *Parasitic Diseases of Wild Mammals* (ed. Samuel, W.M., Pybus, M.J. and Kocan, K.K.), pp. 150–192. Iowa State University Press, Iowa, USA.
- Kharchenko, V. A., Kornyshev, V. V., Varodi, E. I. and Malega, O. M. (2008). Occurrence of *Echinococcus multilocularis* (Cestoda, Taeniidae) in red foxes (*Vulpes vulpes*) from Western Ukraine. *Acta Parasitologica* 53, 36–40.
- Lind, E. O., Juremalm, M., Christensson, D., Widgren, S., Hallgren, G., Ågren, E., Uhlhorn, H. and Lindberg, A. (2011). First detection of *Echinococcus multilocularis* in Sweden, February to March 2011. *Euro Surveillance* 16, 696–705.
- Macdonald, D. (1977). On food preference in the red fox. *Mammal Review* 7, 7–23.
- Maddalena, T., Maurizio, R. and Moretti, M. (2000). Zone di contatto fra *Talpa caeca* Savi e *Talpa europaea* L. in Val Leventina, Valle di Blenio, Val Mesolcina, e Val San Giacomo (Cantoni Ticino e Grigioni, Svizzera / provincia di Sondrio, Italia). *Bollettino della Società Ticinese di Scienze Naturali* 88, 13–18.
- Magi, M., Macchioni, F., Dell'Omodarme, M., Prati, M., Calderini, P., Gabrielli, S., Iori, A. and Cancrini, G. (2009). Endoparasites of red fox (*Vulpes vulpes*) in central Italy. *Journal of Wildlife Diseases* 45, 881–885.
- Manfredi, M., Genchi, C., Deplazes, P., Trevisiol, K. and Fraquelli, C. (2002). *Echinococcus multilocularis* infection in red foxes in Italy. *Veterinary Record* 150, 757.
- Mathis, A., Deplazes, P. and Eckert, J. (1996). An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. *Journal of Helminthology* 70, 219–222.
- Meia, J.-S. and Weber, J.-M. (1995). Home ranges and movements of red foxes in central Europe: stability despite environmental changes. *Canadian Journal of Zoology* 73, 1960–1966.
- Moks, E., Saarma, U. and Valdmann, H. (2005). *Echinococcus multilocularis* in Estonia. *Emerging Infectious Diseases* 11, 1973–1974.
- Nonaka, N., Sano, T., Inoue, T., Teresa Armua, M., Fukui, D., Katakura, K. and Oku, Y. (2009). Multiplex PCR system for identifying the carnivore origins of faeces for an epidemiological study on *Echinococcus multilocularis* in Hokkaido, Japan. *Parasitology Research* 106, 75–83.
- Rajković-Janje, R., Marinculić, A., Bosnić, S., Benić, M., Vinković, B. and Mihaljević, Ž. (2002). Prevalence and seasonal distribution of helminth parasites in red foxes (*Vulpes vulpes*) from the Zagreb County (Croatia). *Zeitschrift für Jagdwissenschaft* 48, 151–160.
- Raoul, F., Deplazes, P., Rieffel, D., Lambert, J.-C. and Giraudoux, P. (2010). Predator dietary response to prey density variation and consequences for cestode transmission. *Oecologia* 164, 129–139.
- Rataj, A. V., Bidovec, A., Žele, D. and Vengušt, G. (2010). *Echinococcus multilocularis* in the red fox (*Vulpes vulpes*) in Slovenia. *European Journal of Wildlife Research* 56, 819–822.
- Rausch, R. L. (1967). On the ecology and distribution of *Echinococcus* spp. (Cestoda: Taeniidae), and characteristics of their development in the intermediate host. *Annales de Parasitologie Humaine et Comparée* 42, 19–63.
- Reperant, L., Hegglin, D., Fischer, C., Kohler, L., Weber, J.-M. and Deplazes, P. (2007). Influence of urbanization on the epidemiology of intestinal helminths of the red fox (*Vulpes vulpes*) in Geneva, Switzerland. *Parasitology Research* 101, 605–611.
- Reperant, L., Hegglin, D., Tanner, I., Fischer, C. and Deplazes, P. (2009). Rodents as shared indicators for zoonotic parasites of carnivores in urban environments. *Parasitology* 136, 329–337.
- Rietschel, G. (1981). Beitrag zur Kenntnis von *Taenia crassiceps* (Zeder, 1800) Rudolphi, 1810 (Cestoda, Taeniidae). *Zeitschrift für Parasitenkunde* 65, 309–315.
- Said-Ali, Z., Grenouillet, F., Knapp, J., Bresson-Hadni, S., Vuitton, D. A., Raoul, F., Richou, C., Millon, L. and Giraudoux, P. (2013). Detecting nested clusters of human alveolar echinococcosis. *Parasitology* 140, 1693–1700.
- Schaefer, O. (1987). *Die Metacestoden der Kleinsäuger (Insectivora und Rodentia) und ihre Wirtsarten Verbreitung und Häufigkeit im Kanton Thurgau (Schweiz)*. Ph.D. thesis, Phil. II. University of Zurich, Zurich, Switzerland.
- Schneider, R., Aspöck, H. and Auer, H. (2013). Unexpected increase of alveolar echinococcosis, Austria, 2011. *Emerging Infectious Diseases* 19, 475–477.
- Schweiger, A., Ammann, R. W., Candinas, D., Clavien, P.-A., Eckert, J., Gottstein, B., Halkic, N., Muellhaupt, B., Prinz, B. M., Reichen, J., Tarr, P. E., Torgerson, P. R. and Deplazes, P. (2007). Human alveolar echinococcosis after fox population increase, Switzerland. *Emerging Infectious Diseases* 13, 878–882.
- Sikó, S. B., Deplazes, P., Ceica, C., Tivadar, C., Bogolin, I., Popescu, S. and Cozma, V. (2011). *Echinococcus multilocularis* in south-eastern Europe (Romania). *Parasitology Research* 108, 1093–1097.
- Sommer, R. and Nadachowski, A. (2006). Glacial refugia of mammals in Europe: evidence from fossil records. *Mammal Review* 36, 251–265.
- Sreter, T., Szell, Z., Egyed, Z. and Varga, I. (2003). *Echinococcus multilocularis*: an emerging pathogen in Hungary and Central Eastern Europe? *Emerging Infectious Diseases* 9, 384–386.
- Staubach, C., Hoffmann, L., Schmid, V. J., Ziller, M., Tackmann, K. and Conraths, F. J. (2011). Bayesian space-time analysis of *Echinococcus multilocularis*-infections in foxes. *Veterinary Parasitology* 179, 77–83.
- Štefanić, S., Shaikenov, B., Deplazes, P., Dinkel, A., Torgerson, P. and Mathis, A. (2004). Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* (“sheep strain”) in naturally infected dogs. *Parasitology Research* 92, 347–351.
- Stieger, C., Hegglin, D., Schwarzenbach, G., Mathis, A. and Deplazes, P. (2002). Spatial and temporal aspects of urban transmission of *Echinococcus multilocularis*. *Parasitology* 124, 631–640.
- Takumi, K., de Vries, A., Chu, M. L., Mulder, J., Teunis, P. and van der Giessen, J. (2008). Evidence for an increasing presence of

- Echinococcus multilocularis* in foxes in the Netherlands. *International Journal for Parasitology* 38, 571–578.
- Tanner, F., Hegglin, D., Thoma, R., Brosi, G. and Deplazes, P. (2006). *Echinococcus multilocularis* in Grisons: distribution in foxes and presence of potential intermediate hosts. *Schweizer Archiv für Tierheilkunde* 148, 501–510.
- Trachsel, D., Deplazes, P. and Mathis, A. (2007). Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. *Parasitology* 134, 911–920.
- Trewhella, W., Harris, S. and McAllister, F. (1988). Dispersal distance, home-range size and population density in the red fox (*Vulpes vulpes*): a quantitative analysis. *Journal of Applied Ecology* 25, 423–434.
- Van Gucht, S., Van Den Berge, K., Quataert, P., Verschelde, P. and Le Roux, I. (2010). No emergence of *Echinococcus multilocularis* in foxes in Flanders and Brussels anno 2007–2008. *Zoonoses and Public Health* 57, e65–e70.
- Veit, P., Bilger, B., Schad, V., Schäfer, J., Frank, W. and Lucius, R. (1995). Influence of environmental factors on the infectivity of *Echinococcus multilocularis* eggs. *Parasitology* 110, 79–86.
- Weber, J. M. and Aubry, S. (2009). Predation by foxes, *Vulpes vulpes*, on the fossorial form of the water vole, *Arvicola terrestris scherman*, in western Switzerland. *Journal of Zoology* 229, 553–559.
- Wilson, D. E. and Reeder, D. M. (2005). *Mammal Species of the World: A Taxonomic and Geographic Reference*, 3rd Edn. Johns Hopkins University Press, Baltimore, MD, USA.

4. DANKSAGUNG

Firstly, I would like to thank Prof. Dr. Peter Deplazes for believing in me and for all the knowledge and guidance provided throughout the past two years.

My big acknowledgements to Daniel Hegglin, great supervisor and friend, for the all the help, the endless debates and the time to listen, especially when things were not going according to plan. It was an honor.

To all the people at the Institute of Parasitology in Zurich who somehow made this work possible. I would like to highlight Teresa Armua and Katja Huggel who always found the time for some tips and small lessons on lab work.

To Philip Staub and Sarah Stephan for helping me with the necropsies. To Gieri Derungs for the endless support and motivation while on the field. And to Olivia Beerli, the new acquisition of the Echino-Team, who I know will continue the project with enthusiasm and endless good mood.

To all the doctoral and PhD students that passed by the library (Jacqi, Lenka, Laura, Hitsch, Ramon, Steffi, Andrea, Sam, Francesca, Sabine).

To my friends.

And finally to my parents.

5. CURRICULUM VITAE

Surnames, Name	Ribeiro Almeida Guerra, Diogo
Birthday	06/01/1988
Place of Birth	Lisbon, Portugal
Nationality	Portuguese

Education & Professional Experience

2003-2006	High School Education, Natural Sciences Background – Colégio Moderno, Lisbon, Portugal
2006-2012	Integrated Master's in Veterinary Medicine – Faculty of Veterinary Medicine, Technical University of Lisbon, Lisbon, Portugal
2008-2010	Student volunteer at LX CRAS – Lisbon Wildlife Rescue Centre
09/2011 - 3/2012	Veterinary Parasitology Internship - (1) the Institute of Parasitology, Vetsuisse Faculty, University of Zurich, and (2) the Parasitic Diseases Routine Laboratory, Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal
06/2012 – 05/2014	Doctoral Thesis at the Institute of Parasitology, Vetsuisse Faculty, University of Zurich Scientific Supervisor: Dr. sc. nat. Daniel Hegglin, Director: Prof. Dr. Peter Deplazes